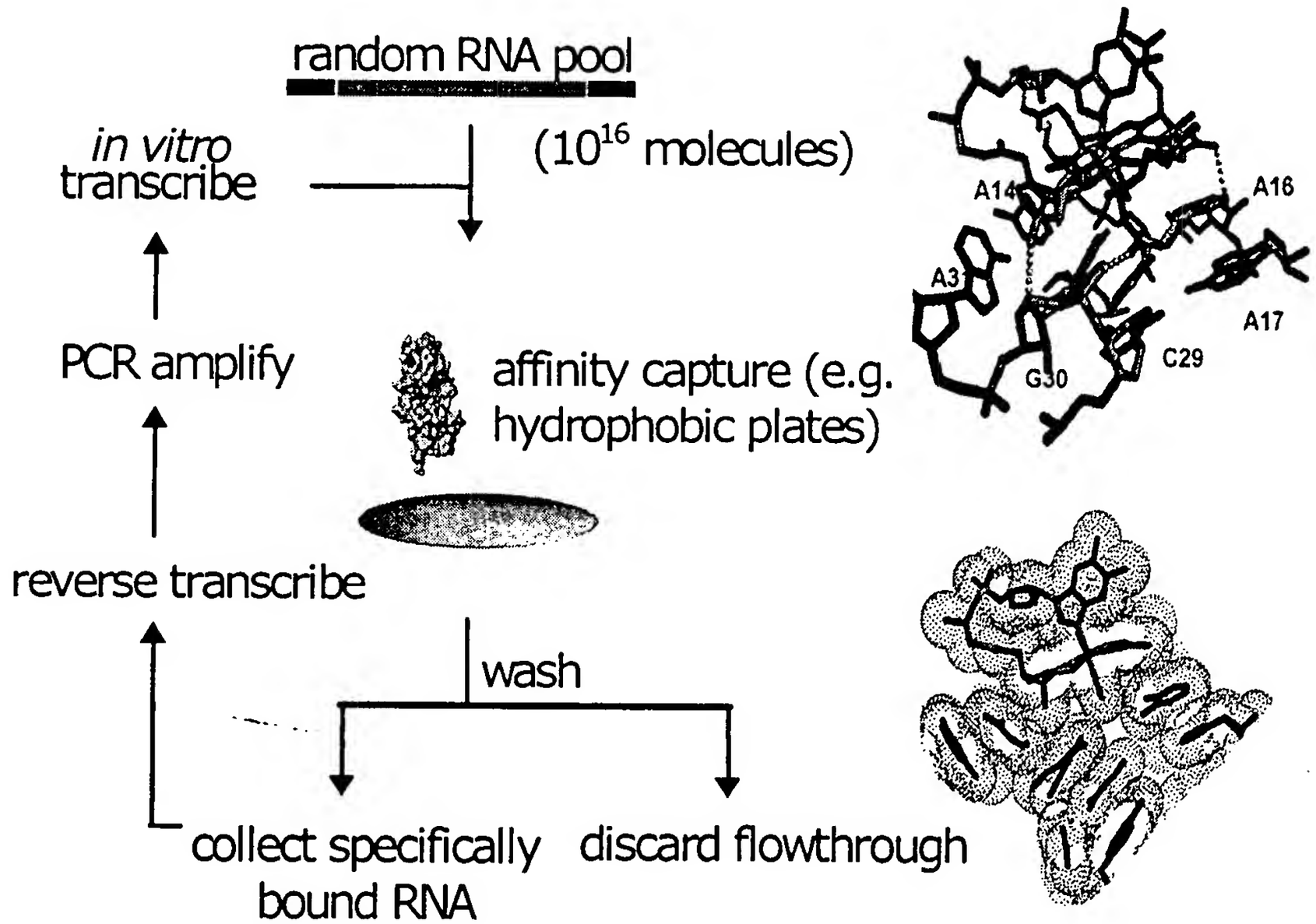

random RNA pool
(10^16 molecules)
in vitro transcribe
PCR amplify
affinity capture (e.g. hydrophobic plates)
reverse transcribe
wash
collect specifically bound RNA
discard flowthrough
A14
A16
A3
A17
G30
C29

Figure 1

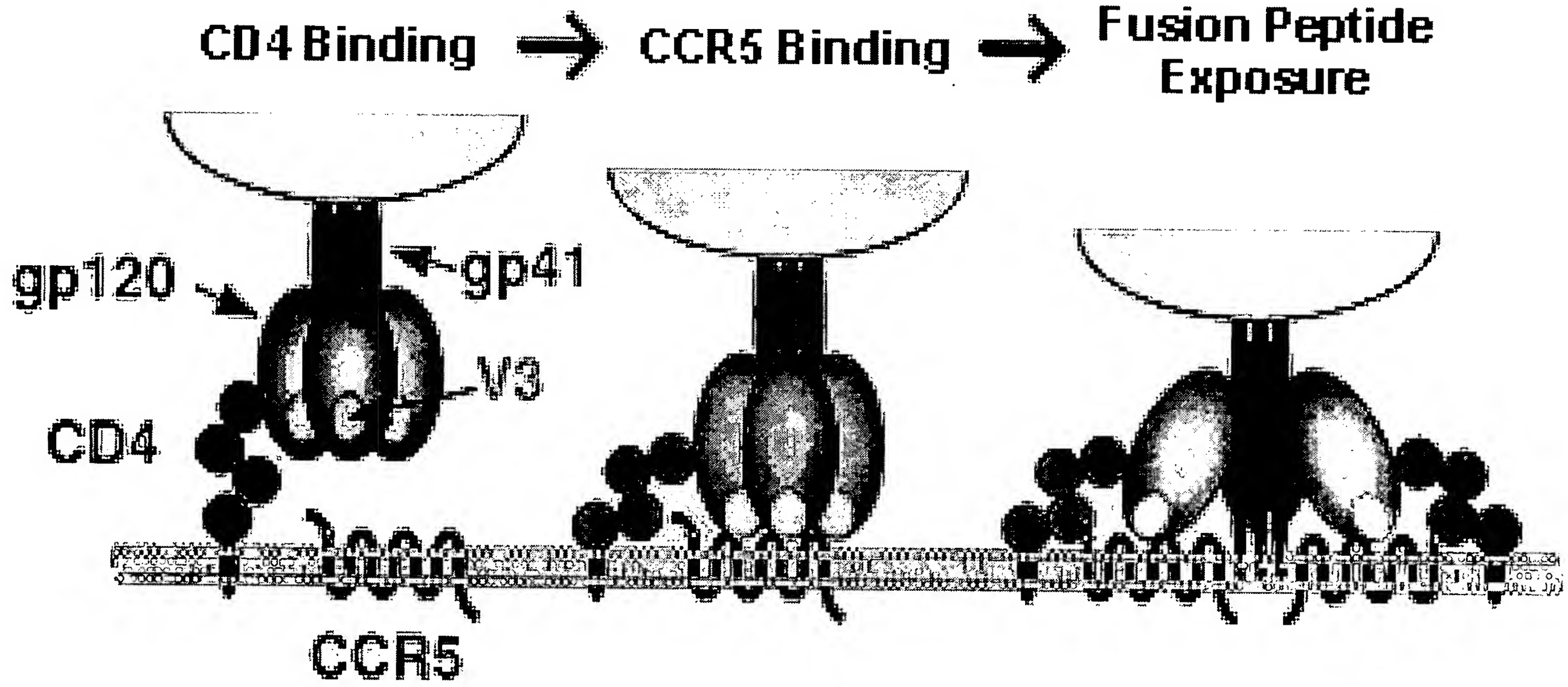

CD4 Binding
CCR5 Binding
Fusion Peptide Exposure
gp120
gp41
V3
CD4
CCR5

Figure 2

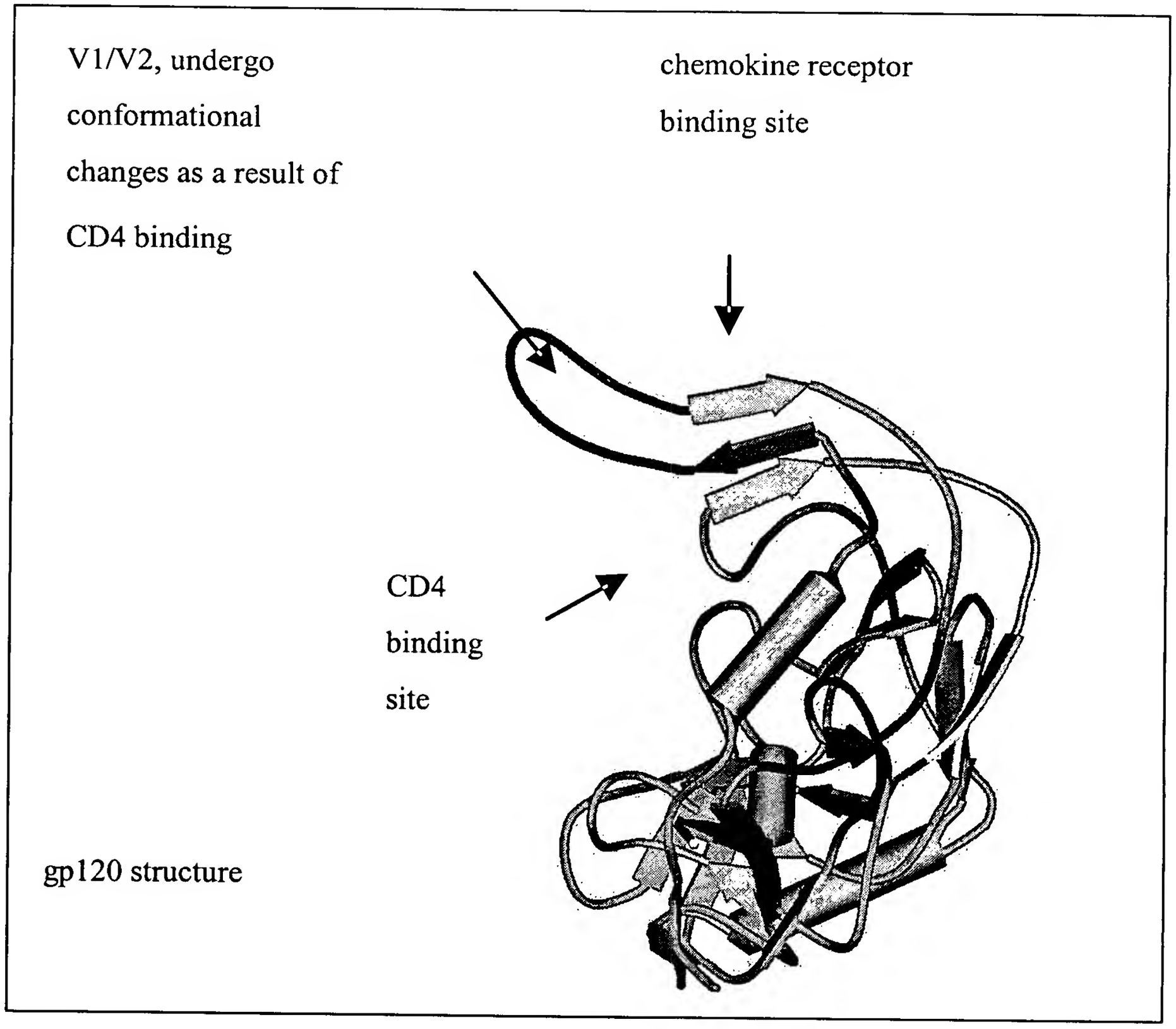

V1/V2, undergo conformational changes as a result of CD4 binding
chemokine receptor binding site
CD4 binding site
gp120 structure

Figure 3

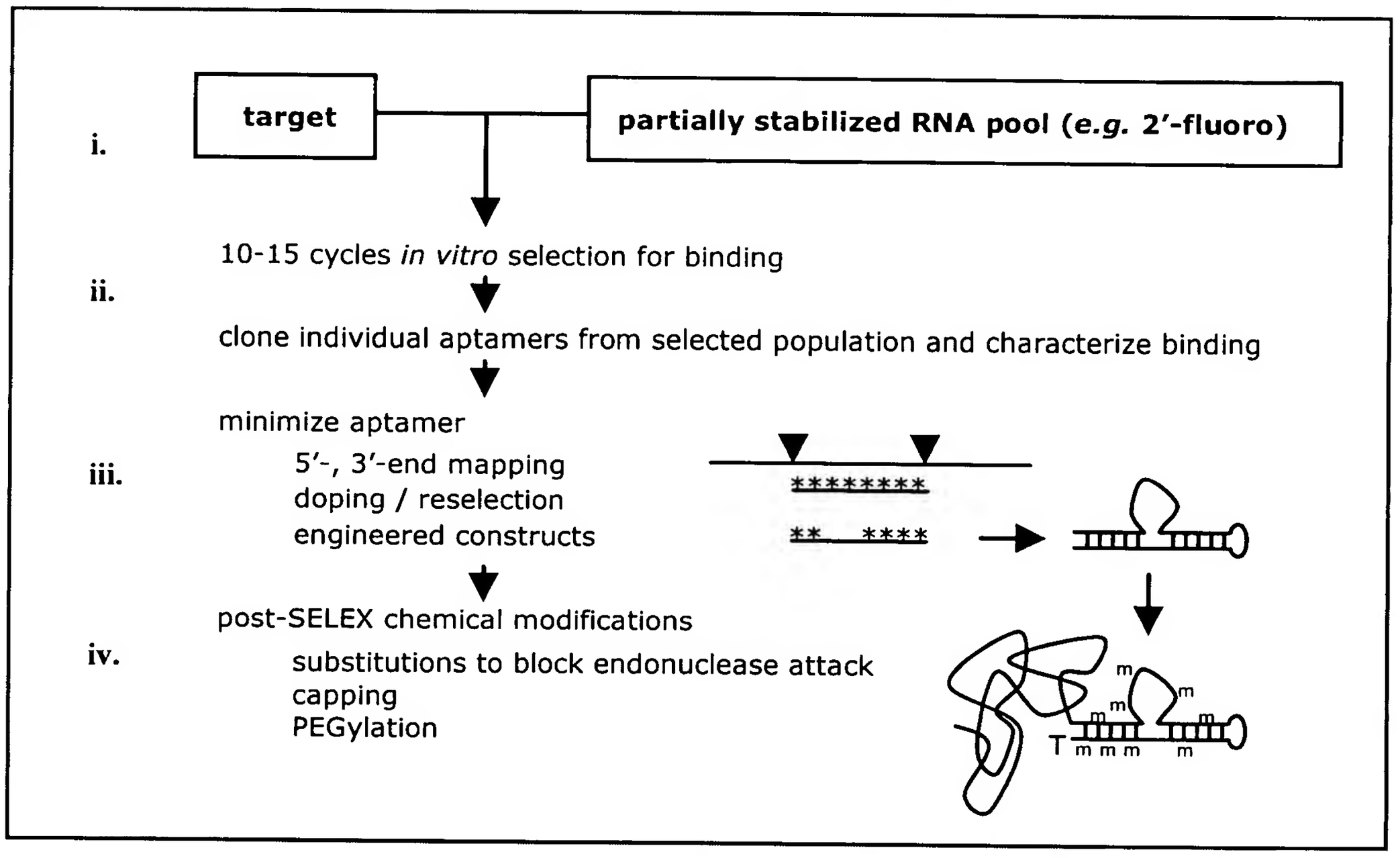

i.
target
partially stabilized RNA pool (e.g. 2'-fluoro)
ii.
10-15 cycles in vitro selection for binding
clone individual aptamers from selected population and characterize binding
iii.
minimize aptamer
5'-, 3'-end mapping
doping / reselection
engineered constructs
iv.
post-SELEX chemical modifications
substitutions to block endonuclease attack
capping
PEGylation
m
T

Figure 4

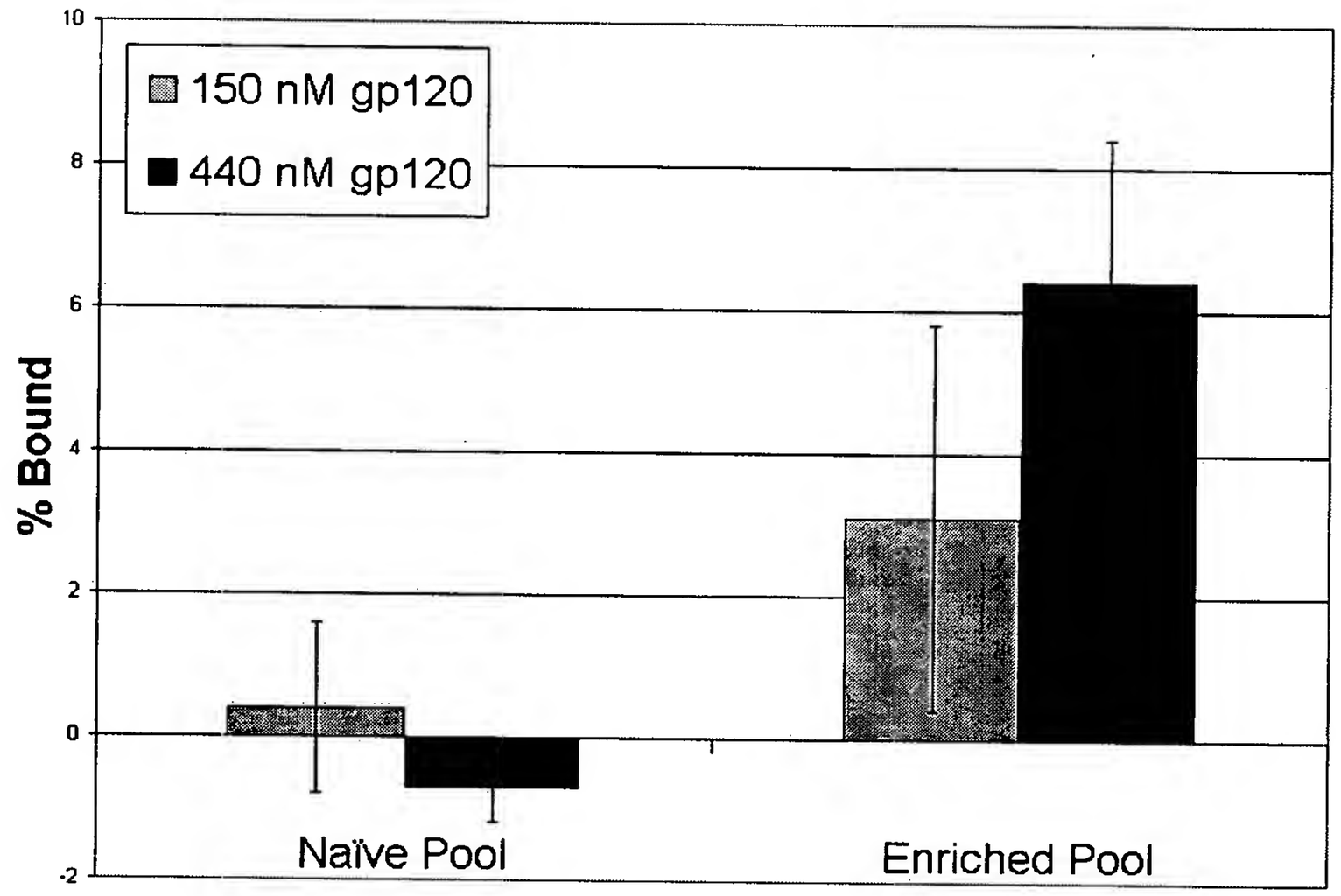

10
8
6
4
2
0
-2
% Bound
150 nM gp120
440 nM gp120
Naïve Pool
Enriched Pool

Figure 5

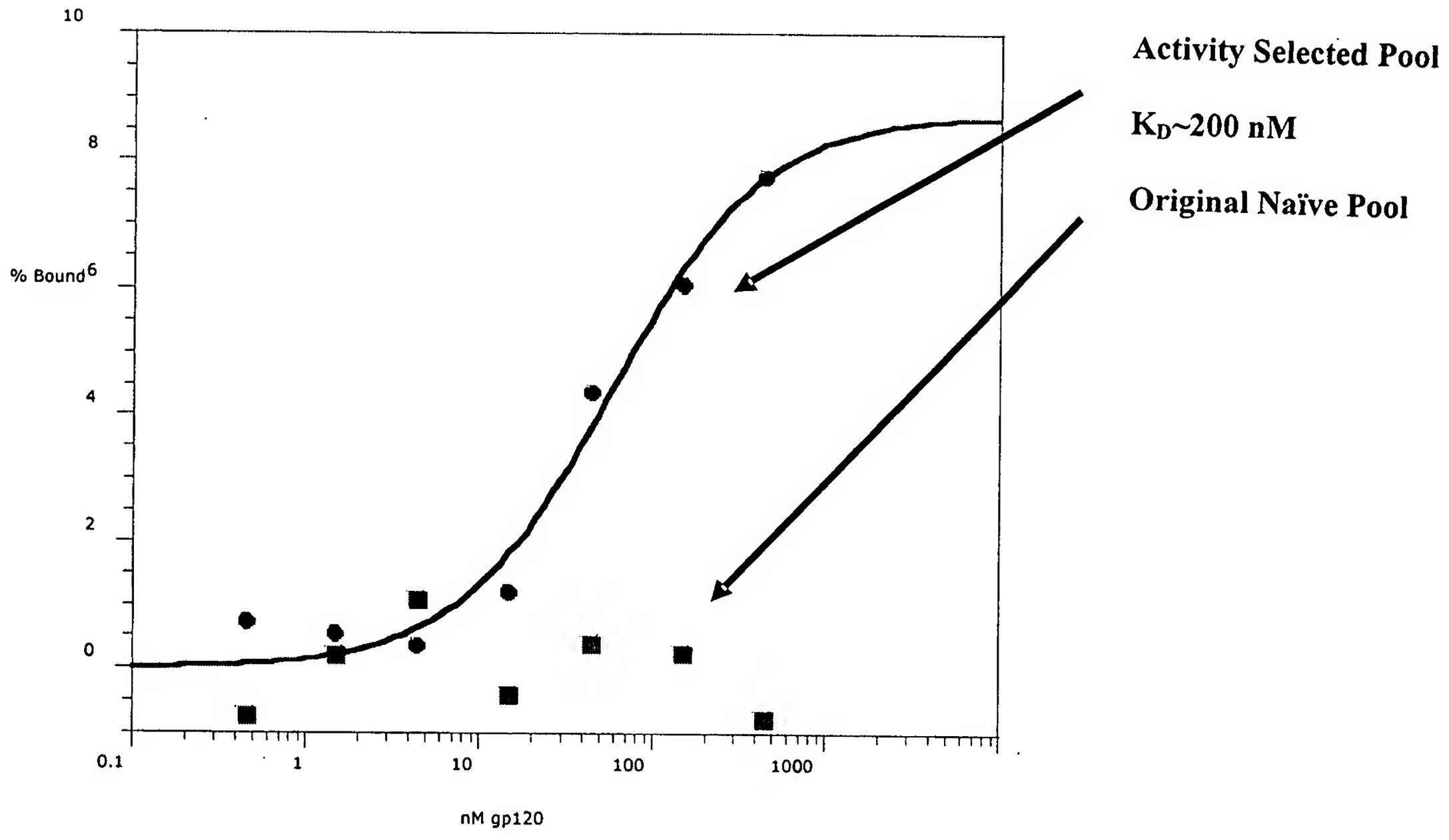

10
8
% Bound
6
4
2
0
0.1
1
10
100
1000
nM gp120
Activity Selected Pool
K_D~200 nM
Original Naïve Pool

Figure 6

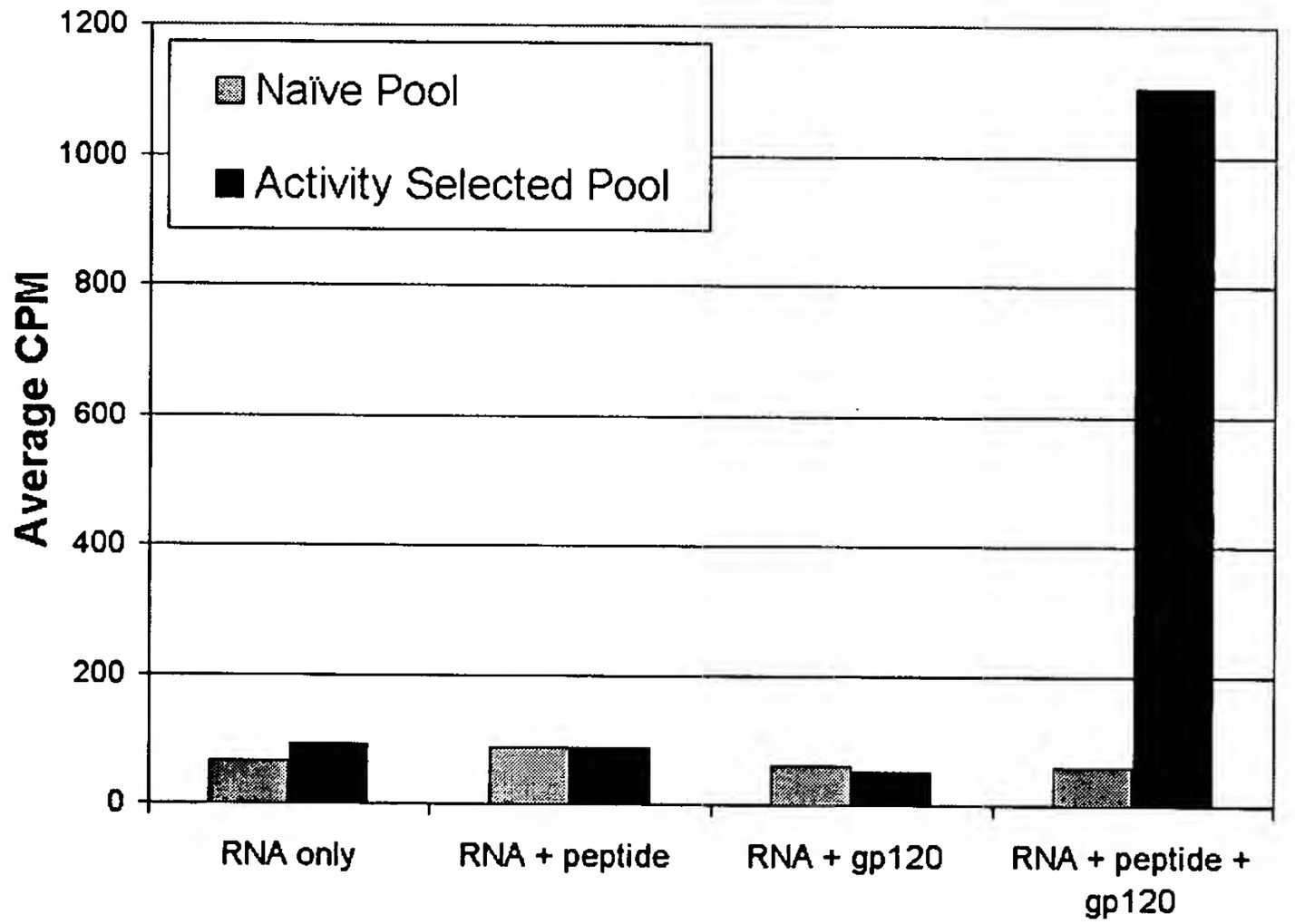
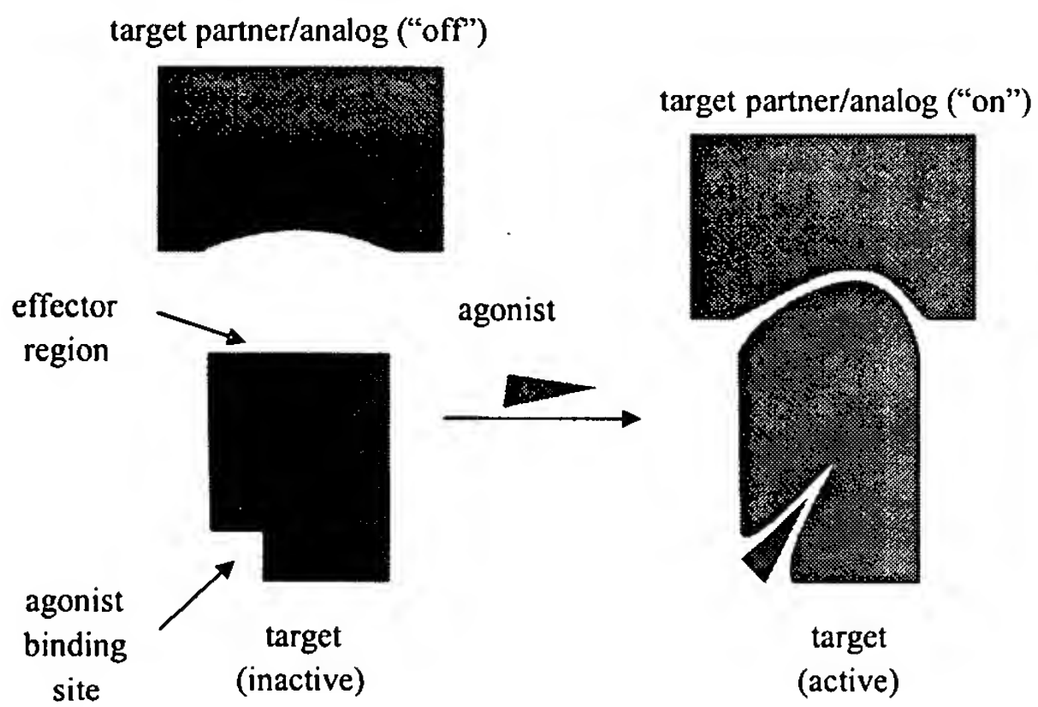

1200
1000
800
600
400
200
0
Average CPM
Naïve Pool
Activity Selected Pool
RNA only
RNA + peptide
RNA + gp120
RNA + peptide + gp120

Figure 7

gp120 = target
CD4 = agonist
CCR5/CXCR4 = target partner
17b = target partner analog
agonist function = alter conformation of chemokine receptor binding site on gp120 to facilitate an immune response
(e.g., generation of 17b-like antibodies)



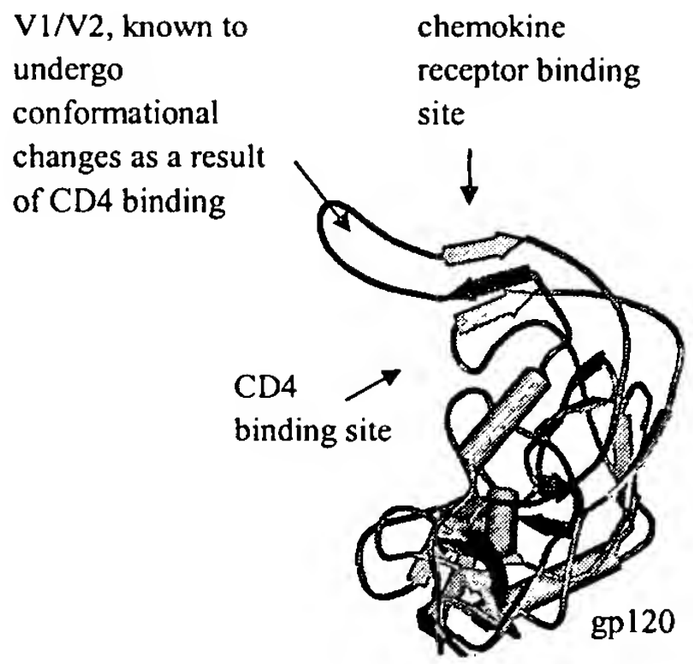


Figure 8

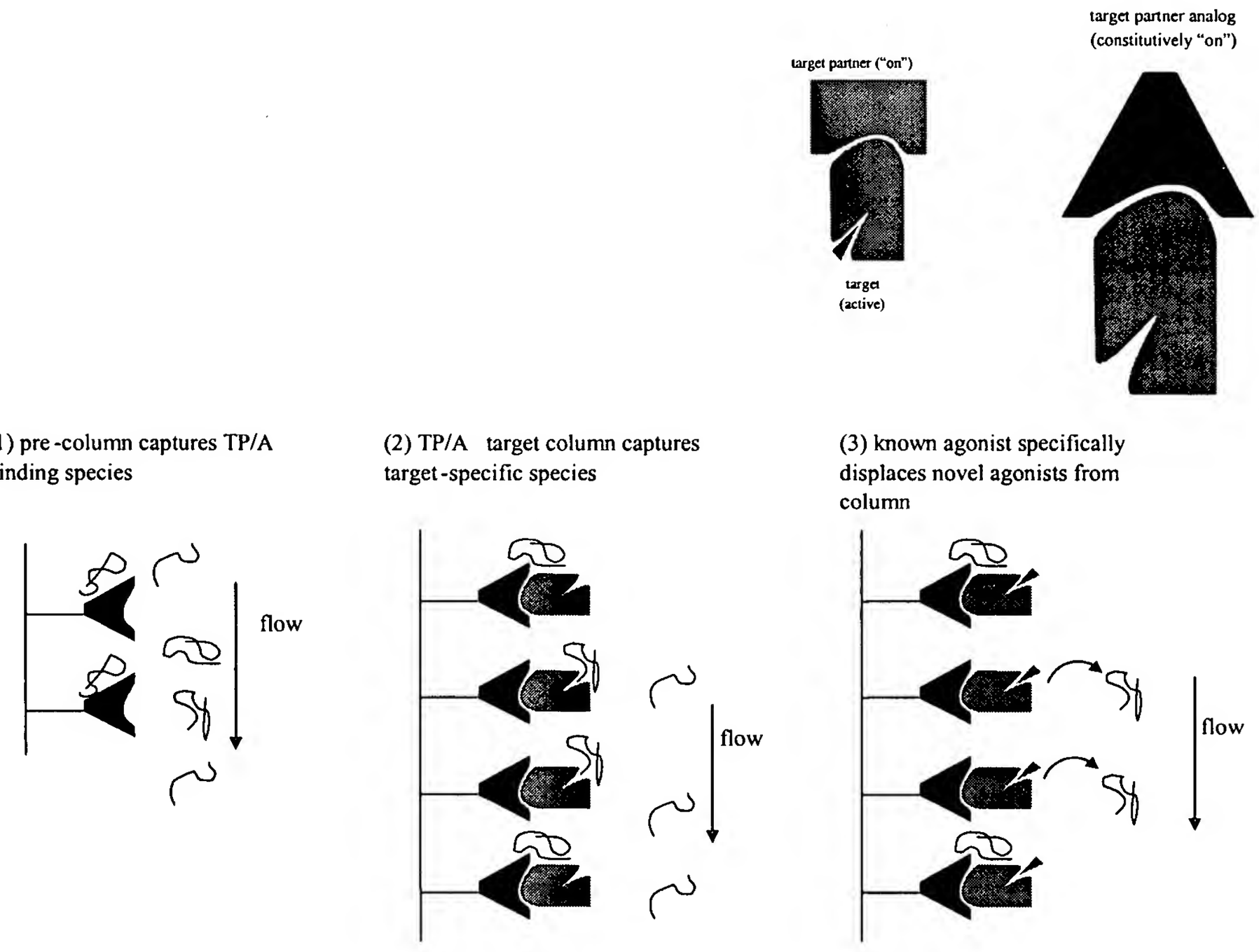
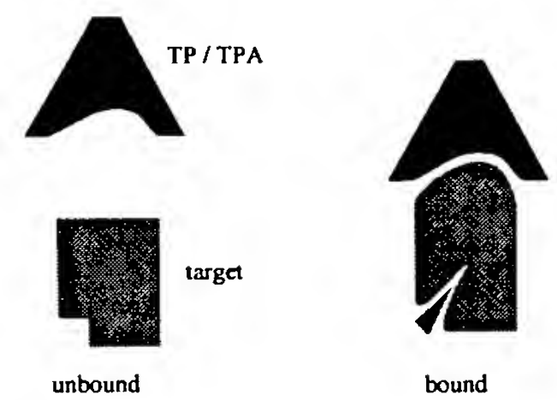

target partner ("on")
target partner analog
(constitutively "on")
target
(active)
(1) pre-column captures TP/A binding species
(2) TP/A target column captures target-specific species
(3) known agonist specifically displaces novel agonists from column
flow
flow
flow

Figure 9


TP / TPA
target
unbound
bound

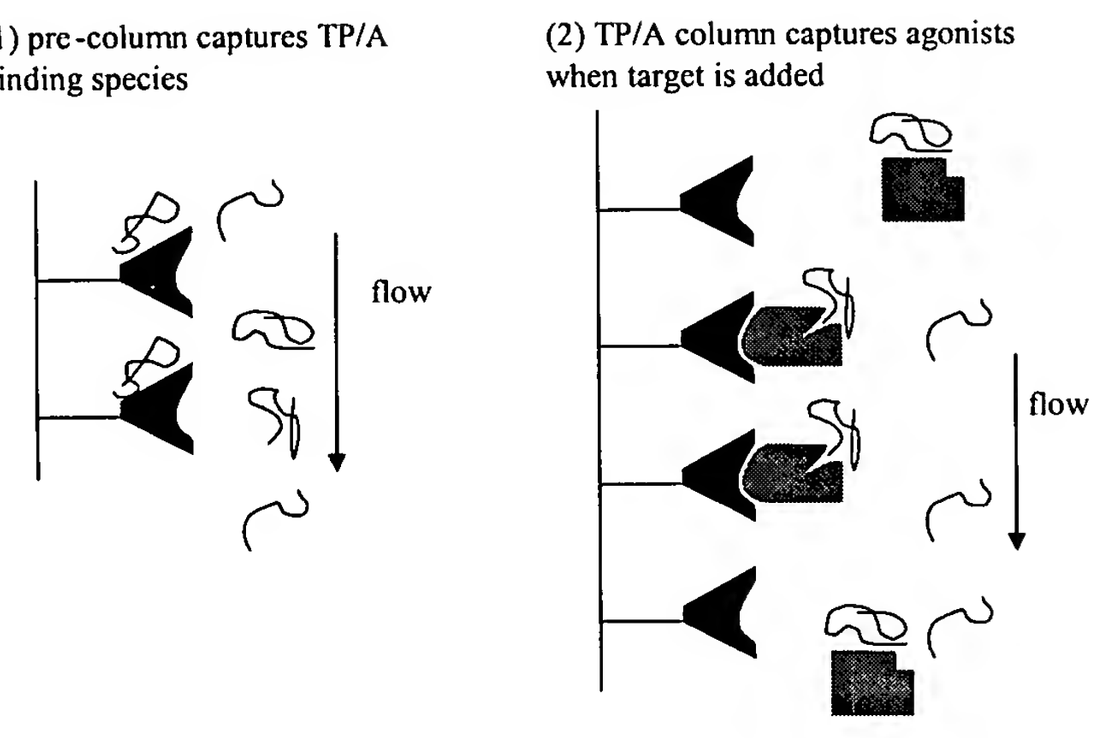

(1) pre-column captures TP/A binding species
flow
(2) TP/A column captures agonists when target is added
flow

Figure 10

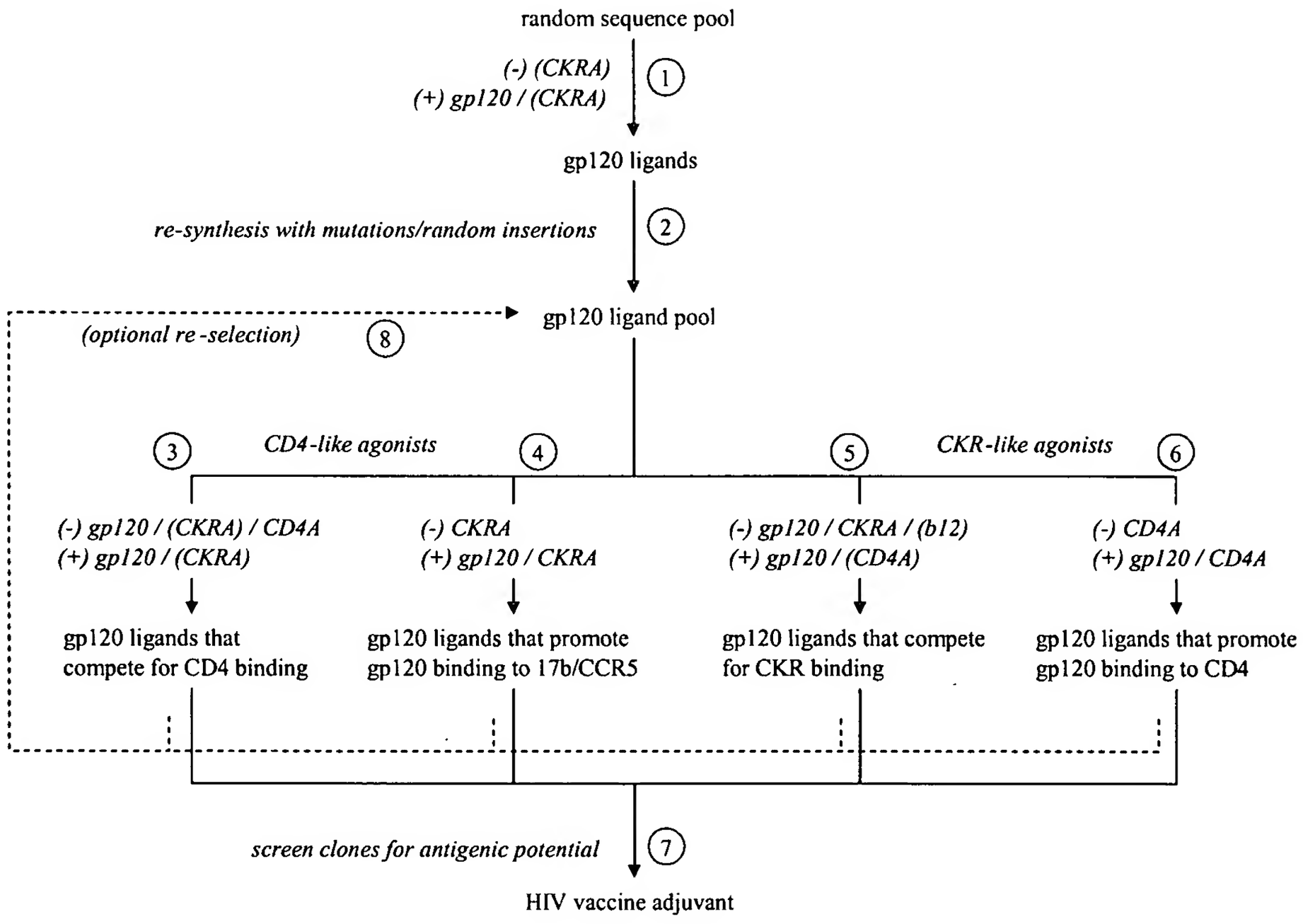
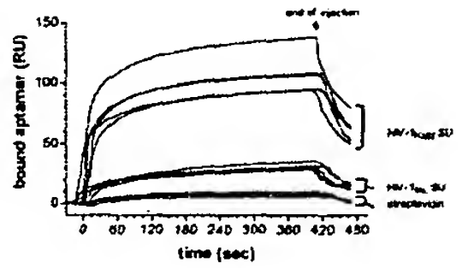

random sequence pool
(-) (CKRA)
(+) gp120 / (CKRA)
1
gp120 ligands
re-synthesis with mutations/random insertions
2
gp120 ligand pool
(optional re-selection)
8
3
CD4-like agonists
4
5
CKR-like agonists
6
(-) gp120 / (CKRA) / CD4A
(+) gp120 / (CKRA)
(-) CKRA
(+) gp120 / CKRA
(-) gp120 / CKRA / (b12)
(+) gp120 / (CD4A)
(-) CD4A
(+) gp120 / CD4A
gp120 ligands that compete for CD4 binding
gp120 ligands that promote gp120 binding to 17b/CCR5
gp120 ligands that compete for CKR binding
gp120 ligands that promote gp120 binding to CD4
screen clones for antigenic potential
7
HIV vaccine adjuvant

Figure 11

Isolate clones, assay for binding



Sequence clones

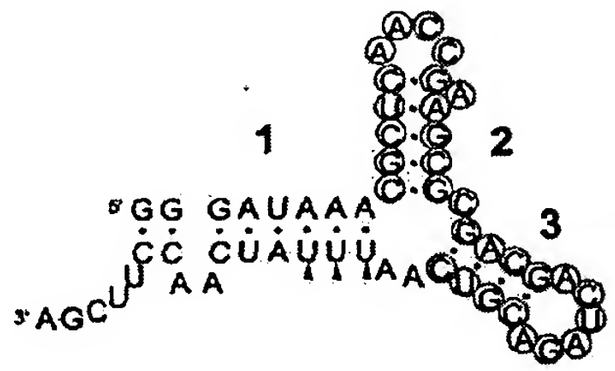
GGGAGACAAGAAUAAACGCUCAACCGAAGCGCGACGACUAGACGUCAAUUUAUCAACCUUCGACAGGAGGCUCACAACAGGC
(SEQ ID NO: 227)

Truncation analysis
to define 5'- and 3'-ends

AUAAACGCUCAACCGAAGCGCGACGACUAGACGUCAAUUUAU (SEQ ID NO: 228)

Define structure by synthesis of variants or *in vitro* phylogenetic methods

(SEQ ID NO: 226)



Chemically synthesize diverse pool based on aptamer structure

GGACACAUACUCUACA-N20-gggauaaacgcucaaccgaagcgcgacgacuagacgucaauuuaucaaccuucga-N20-UUAACCCAGCACGCCUCGUA
(SEQ ID NO: 229) (SEQ ID NO: 230) (SEQ ID NO: 231)

A,C,G,U: specified nucleotide (U→T for DNA synthesis)
N: equal proportions of A,C,G,U
a,c,g,u: 85% specified nucleotide, 5% of each other nucleotide

Append random sequence tags to existing aptamers by PCR or ligation

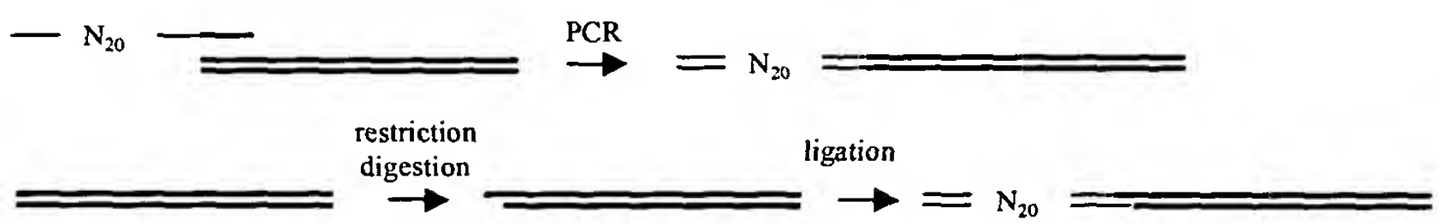


Figure 12